MicroRNAs as promising biomarkers and therapeutic tools in hereditary hemorrhagic telangiectasia

Hereditary hemorrhagic telangiectasia (HHT) is a genetic disorder that results in potentially fatal blood vessel abnormalities. Currently, there is no cure or universally effective treatment for the condition, which remains underfunded and underdiagnosed. MicroRNAs have emerged as new biomarkers for human diseases and hold great promise for the improved diagnosis and treatment of HHT. A recent review publication by Anthony Cannavicci, PhD candidate at the Institute of Medical Science, University of Toronto, discusses the utility of microRNAs as circulating biomarkers and potential therapeutic targets in HHT. His work underscores the importance of studying these non-coding RNAs to further understand disease pathogenesis.

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Hereditary hemorrhagic telangiectasia (HHT) is a rare genetic disorder characterised by abnormal blood vessel formation. Current prevalence is estimated between 1 in 5,000 to 8,000 persons globally. Individuals with HHT tend to develop direct connections between arteries and veins that lack capillary networks, resulting in a fragile vessel susceptible to rupture and bleeding. These abnormal blood vessels are known as telangiectasias if they involve small blood vessels, and arteriovenous malformations (AVMs) if they involve larger blood vessels. Telangiectasias tend to occur near the surface of the skin or the mucous membranes that line the nose and gastrointestinal tract, while AVMs are typically seen in the lungs, brain, spinal cord, or liver.

Recurrent and spontaneous nosebleeds are the most common symptom of HHT, and gastrointestinal bleeding can be another possible indicator of the condition. AVMs within the brain, lungs, liver, and spine often do not display any warning signs before rupturing, which can cause life-threatening bleeding and numerous complications. Currently, there is no cure for HHT and treatment for the condition generally is focused on managing the symptoms. MicroRNAs (miRs) have recently emerged as biomarkers and therapeutic targets for a variety of human diseases, but the study of these molecules in HHT has been limited. Anthony Cannavicci, a PhD candidate at the Institute of Medical Science, University of Toronto, hopes to change this. In his review paper, he highlights the importance of studying miR dysregulation in HHT. This work has the potential to identify unique therapeutic targets and aid the development of novel diagnostic tools for HHT.

CHARACTERISTICS OF HHT

HHT is inherited in an autosomal dominant fashion with varying penetrance and expressivity. There are at least three types of HHT differentiated primarily by the genetic cause, signs, and symptoms. Type 1 (HHT1) is caused by mutations in the *ENG* gene on chromosome 9, and type 2 (HHT2) is caused by mutations in the *ACVRL1* gene located on chromosome 12. A smaller percentage of individuals have a mutation in the *SMAD4* gene on chromosome 18, which causes a juvenile polyposis/HHT overlap syndrome.

Each of the three genes implicated in HHT encodes a protein involved in the transforming growth factor beta (TGF-β) / bone morphogenetic protein (BMP) signalling pathway. This signalling pathway is important in the regulation of many cellular processes, including growth, differentiation, apoptosis, and vascular remodelling and maintenance. In patients with HHT, mutations in either *ENG, ACVRL1* and *SMAD4* lead to a reduction of functional protein products, known as haploinsufficiency. Abnormalities in their protein products cause the malfunction of TGF-β/BMP signalling in endothelial cells, resulting in the defective formation of blood vessels. Interestingly, disease severity and its clinical manifestations vary greatly between patients and even among members of the same family. This suggests that genetic mutations alone are not entirely responsible for disease characteristics, and other biological factors must be at play.

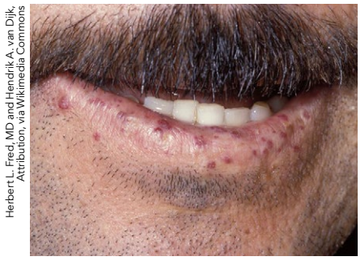
NON-CODING RNAS

Non-coding RNAs (ncRNAs) are RNA molecules that are not translated into protein and include miRs, a class of short non-coding RNAs (~21–25 nucleotides), and long non-coding RNAs (lncRNAs) consisting of more than 200 nucleotides. Acting to regulate gene expression at both the transcriptional (regulating the conversion of DNA to RNA) and post-transcriptional level (regulation at the RNA level before translation into protein), ncRNAs are known to play important roles in almost all cellular processes.

MiRs are the best-studied group of ncRNAs. They were first discovered in 1993 by the Ambros and Ruvkun groups and have revolutionised the field of molecular biology. Over 2000 miRs have been identified, and it has been suggested that they regulate 30% of known genes. MiRs regulate gene expression by directing their target mRNAs for degradation or translational repression. Over the past decade, it has become clear that miR expression is dysregulated in a wide range of human diseases. MiRs have been extensively studied in oncology, where they were shown to have reliable diagnostic and prognostic attributes and are being pursued as potential therapeutic targets. However, a growing class of miRs known as 'angiomiRs' have also been shown to contribute to vascular diseases and could therefore play a role in HHT pathogenesis.

MiRs AS POTENTIAL BIOMARKERS IN HHT

HHT is an extremely underdiagnosed condition, and without management, it can lead to serious morbidity and mortality. Currently, HHT is diagnosed in combination by a clinical criterion known as the Curaçao criteria alongside genetic testing. However, many HHT patients do not present a clear diagnosis or do not show pathogenic mutations in known HHT genes. Furthermore, approximately 50% of patients develop pulmonary AVMs, 80% develop hepatic AVMs, 10% develop cerebral AVMs, and 1% develop spinal AVMs, all of which can lead to life-threatening bleeding and other complications. Screening patients with HHT is therefore crucial, but current diagnostic screens are costly, inaccessible, and expose patients to unhealthy doses of radiation. This has prompted the need to identify additional biomarkers of the disease.



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The most common symptom of HHT are nosebleeds, but many individuals also experience skin lesions on the lips, nose and fingers.

Several research groups have shown that the detection of dysregulated miRs in blood plasma may be a promising diagnostic tool to identify clinical manifestations in HHT patients. One such study by Zhang *et al.* (2013) found that circulating miR-210 levels were significantly elevated in plasma from HHT patients with PAVMs, but not in patients without PAVMs. If validated in a larger clinically relevant sample size, miR-210 could be a novel biomarker for the detection of PAVMs in HHT.

A later study by Tabruyn *et al.* (2013) also supports the idea that detection of circulating miRs could help in the screening of HHT patients. They found that plasma levels of miR-27a were significantly increased, whilst levels of miR-205 were significantly reduced in both HHT1 and HHT2 patients, compared to healthy controls. Interestingly, they demonstrated that miR-205 expression modulates the TGF-β pathway, providing a functional link between dysregulated expression of miR-205 and HHT. Consequently, miR-205 may not only be a marker of HHT, but could also be a potential therapeutic target. Recently, Ruiz Llorente *et al.* (2020) demonstrated that circulating miRs could distinguish between HHT1 or HHT2 patients, which could greatly improve the diagnostic process. In this study, miR-370, which is predicted to target *ENG*, was downregulated in HHT1, but not HHT2 patients, whilst miR-10a, which is predicted to target *ACVRL1*, was upregulated in HHT2, but not HHT1 patients. With further research, circulating miRs could potentially provide a rapid, inexpensive, safe, and relatively non-invasive screening test for the diagnosis of AVMs and HHT.

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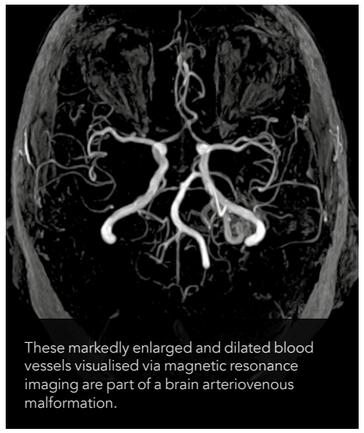
MiRs AS THERAPEUTIC TARGETS IN HHT

Several miRs, termed 'angiomiRs', which are predominately expressed in endothelial cells and are responsible for the regulation of angiogenesis (new blood vessel formation), have been shown to be regulated by and target components of the TGF-β signalling pathway. Given the importance of TGF-β signalling in the pathogenesis of HHT, it seems likely that the dysregulation of angiomiRs plays a role in HHT.

MiR-26, which is enriched in the endothelial lining of the blood vessel wall, has recently been shown to be involved in vascular stability by directly targeting SMAD1, part of a family of proteins that mediate TGF-β signalling. Loss of this miR in a zebrafish model led to an increase in SMAD1, resulting in vascular smooth muscle cell dysregulation and hemorrhage.

Another study demonstrated that overexpression of miR-148b increased endothelial cell migration, proliferation, and angiogenesis by targeting SMAD2



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These markedly enlarged and dilated blood vessels visualised via magnetic resonance imaging are part of a brain arteriovenous malformation.

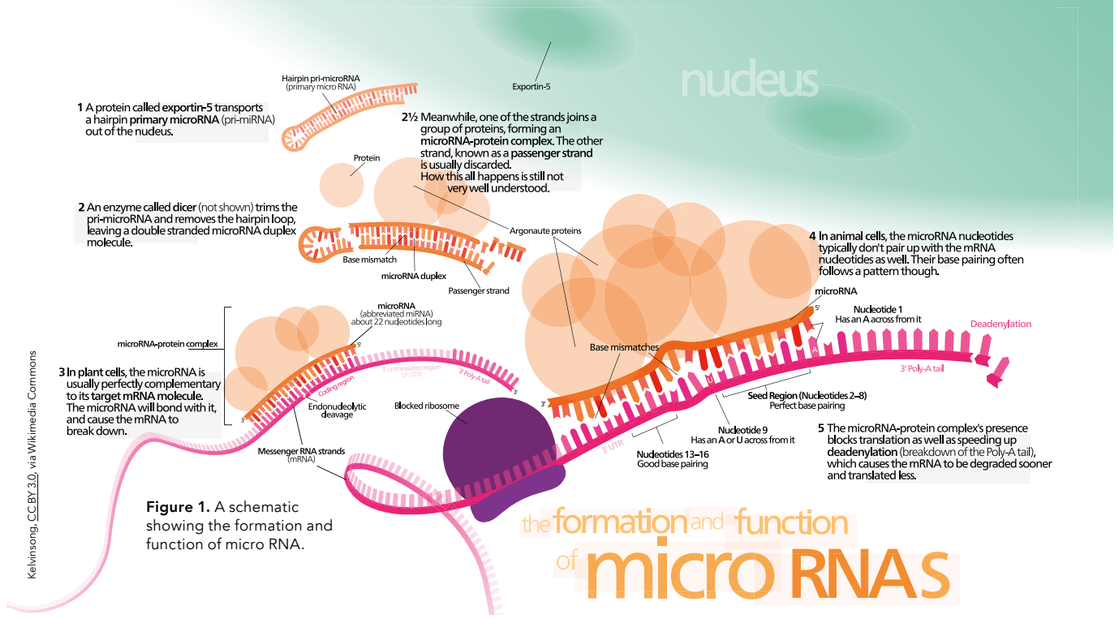

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Figure 1. A schematic showing the formation and function of micro RNA.

and a TFG-β receptor. In a mouse model, delivery of miR-148b mimics promoted wound vascularisation and accelerated wound closure, whilst inhibition of miR-148b impaired wound closure, but was rescued by silencing SMAD2. Decreased levels of miR-361-3p and miR-28-5p, known to target insulin growth factor 1 (IGF-1), have also been found in peripheral blood mononuclear cells (PBMNCs) from HHT patients. The mRNA levels of IGF-1, an important angiogenic factor, was shown to be significantly upregulated in HHT-derived PBMNCs and may be related to the dysregulation of miR-361-3p and miR-28-5p. Although further research is required, this may be an important pathogenic mechanism involved in abnormal blood vessel formation in HHT and could provide a unique miR therapeutic target.

DYSREGULATION OF lncRNAS IN HHT

LncRNAs can be classified according to their genomic location – nuclear and cytoplasmic – and can activate, repress, or otherwise modulate the expression of target genes through various mechanisms. Although only a small number of lncRNAs have been characterised functionally, increasing evidence suggests that they are involved in a variety of cellular functions and could serve as alternative therapeutic targets.

MALAT1, which is amongst the most abundant and highly conserved lncRNA, is highly expressed in endothelial cells and is thought to be involved in the angiogenic response of these cells by promoting cell proliferation and migration under low oxygen conditions (hypoxia). Singh *et al.* (2016) were the first to show differential expression of lncRNAs, including MALAT1, in endothelial cells in response to TGF-β. Given that dysregulated TGF-β signalling is linked to HHT, lncRNAs look likely to play a role in the development of the disease. Indeed, evidence to support this came from the work of Torring *et al.* (2014), which demonstrated that 42 lncRNAs were significantly dysregulated in telangiectasial nasal mucosa compared to non-telangiectasial nasal mucosa from the same HHT patients. Interestingly, these lncRNAs were enriched in HHT-related pathways, such as blood vessel formation and development. Whilst further work is required to gain a deeper understanding of the role of lncRNAs in HHT, it is an exciting area of study for discovering therapeutic targets and for identifying new biomarkers.

Although the exact role of any class of miR has yet to be fully characterised in HHT, it is a field of study that holds great promise. With further research, ncRNAs may prove to have both diagnostic and therapeutic applications for those with HHT.

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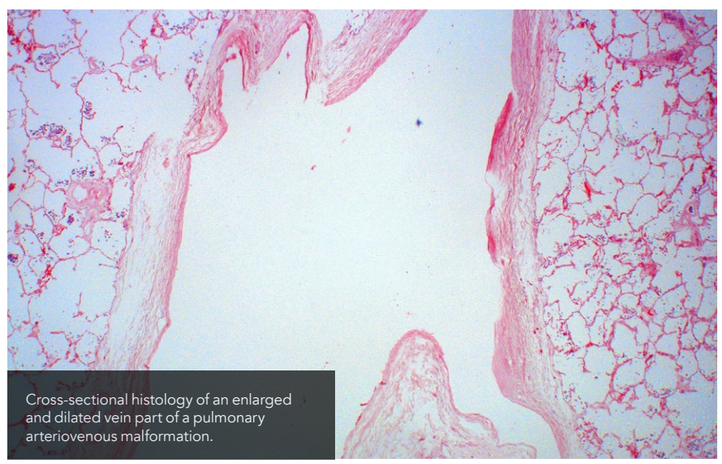

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Cross-sectional histology of an enlarged and dilated vein part of a pulmonary arteriovenous malformation.

Behind the Research

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Research Objectives

Anthony Cannavicci researches microRNA dysregulation in HHT to further elucidate pathogenesis and identify novel diagnostic and therapeutic targets.

Detail

Bio

Anthony Cannavicci is a PhD candidate at the Institute of Medical Science, University of Toronto. He studies microRNA dysregulation in hereditary hemorrhagic telangiectasia (HHT) under the supervision of Dr. Michael Kutryk at St. Michaels's Hospital. Outside of research, Anthony advocates for HHT awareness as president of the charity, HHT Canada.

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Personal Response

What do you see to be the main challenges and risks around developing miR as therapeutics and diagnostic biomarkers for HHT?

"Since the discovery of miRs in 1993, significant strides have been made; in 2002, miRs were first implicated in human disease and in 2008 were first detected in human blood. Presently, miRs are being pursued as therapeutic and diagnostic targets, but have yet to significantly impact clinical practice and care. MiRs are very complex and promiscuous biomolecules that can potentially have tens of targets. This poses a challenge for their utilisation as therapeutic targets in HHT. In the context of enhancing or inhibiting miR function, great care is necessary to ensure off-target effects are mitigated. The improvement of drug delivery technology could greatly enhance the efficacy of miR therapeutics in potentially treating vascular abnormalities found in HHT. MiRs have been shown to be reliable and sensitive non-invasive biomarkers for various diseases and could greatly aid the diagnostic process. MiRs derived from bodily fluids, such as blood plasma or saliva, are in low abundance and technological limitations in miR extraction and purification can pose a challenge in their detection. MiR research and application in HHT is very much still in its infancy and holds great promise. It is paramount that more scientists and clinicians take interest in the potential of studying miRs for further understanding and treating HHT. I am enthusiastic and hopeful that in the near future, miRs can be effective diagnostic and therapeutic targets for numerous diseases, including HHT."